

WE CLAIM:

1 1. A method for individually detecting a plurality of analytes in a
2 single fluid biological sample by assays that include the binding of species in said
3 biological sample to a solid phase that is in contact with a liquid medium in which said
4 solid phase is insoluble and the separation of said solid phase from said liquid medium,
5 said method comprising:

6 6. using as said solid phase a plurality of microparticles of magnetically
7 responsive material each with an assay reagent coupled thereto that is selectively
8 active in an assay for one of said analytes, said microparticles classifiable into
9 groups differing by the value of a selected differentiation parameter whose value
10 is detectable, each group distinguishable from other groups by the value of said
11 parameter and by the assay reagent coupled to the microparticles of said group;

12 12. magnetically separating microparticles in all of said groups from said
13 liquid medium; and

14 14. defining said liquid medium as a first liquid medium, suspending said
15 microparticles separated therefrom in a second liquid medium, analyzing said
16 microparticles in said second liquid medium in accordance with both the value of
17 said selected differentiation parameter and said plurality of assays, thereby
18 achieving individual detection of said analytes in said biological sample.

1 2. A method in accordance with claim 1 in which said selected
2 differentiation parameter is particle size and differentiation among said groups is
3 performed by flow cytometry.

1 3. A method in accordance with claim 1 in which one of said assay
2 reagents coupled to said solid phase is a binding protein specific for one of said analytes,
3 said method includes adding to said first liquid medium a detectable label that binds to
4 said solid phase, and said magnetic separation comprises separating detectable label that
5 is bound to said solid phase from unbound detectable label that is suspended in said first
6 liquid medium.

1 4. A method in accordance with claim 3 in which said detectable label
2 is added to said first liquid medium as a conjugate with an additional quantity of said one

3 analyte, causing said conjugate and said one analyte in said sample to compete for said
4 binding protein in a competitive assay.

1 5. A method in accordance with claim 3 in which said detectable label
2 is added to said first liquid medium or said second liquid medium as a conjugate with an
3 additional quantity of said one analyte after a first incubation period, causing said
4 conjugate to react with those sites of said binding protein not occupied by said one
5 analyte in said sample in a sequential assay.

1 6. A method in accordance with claim 3 in which said binding protein
2 is defined as a first binding protein, and said detectable label is added to said first liquid
3 medium as a conjugate with a second binding protein that is also specific for said one
4 analyte, causing said one analyte to bind to both said first binding protein and said
5 conjugate in a sandwich assay.

1 7. A method in accordance with claim 6 in which said one analyte is
2 an antigen, said first binding protein is a first antibody to said antigen, and said second
3 binding protein is a second antibody to said antigen.

1 8. A method in accordance with claim 1 in which one of said assay
2 reagents is an antigen bound to said solid phase and specific for one of said analytes in
3 said first liquid medium which is an antibody of a selected immunoglobulin class, and
4 said separation comprises separating said microparticles with said one analyte bound
5 thereto from antibodies of said selected immunoglobulin class other than those specific
6 for said bound antigen, in said first liquid medium prior to contacting said microparticles
7 with a second liquid medium containing a detectable label that binds to all members of
8 said selected immunoglobulin class.

1 9. A method in accordance with claim 1 in which analysis of said
2 microparticles is performed by fluorescence detection.

1 10. A method in accordance with claim 1 in which said analytes in said
2 assays are detected by the use of a phycoerythrin-labeled binding member and analysis of
3 said microparticles is performed by detection of fluorescence of said phycoerythrin label.

1 **11.** A method in accordance with claim **2** in which said range is a
2 diameter from about 0.3 micrometers to about 100 micrometers.

1 **12.** A method in accordance with claim **2** in which said range is a
2 diameter of from about 0.5 micrometers to about 40 micrometers.

1 **13.** A method in accordance with claim **2** in which the standard
2 deviation of the particle diameters of each of said subranges is less than one-third of the
3 separation of the mean diameters of adjacent subranges.

1 **14.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is fluorescence decay time, and differentiation among said
3 groups is performed by time-resolved fluorescence detection.

1 **15.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is degree of light scatter.

1 **16.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is intensity of fluorescence.

1 **17.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is a combination of forward light scatter, lateral light scatter,
3 and fluorescence intensity at a plurality of wavelengths.

1 **18.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is absorbance.

1 **19.** A method in accordance with claim **1** in which said selected
2 differentiation parameter is the relative quantities of particles in each group.

1 **20.** A method in accordance with claim **1** in which said microparticles
2 are comprised of a combination of a polymer and a paramagnetic substance.

1 **21.** A composition comprising a plurality of solid-phase assay reagents
2 selectively active in a plurality of assays each for a different analyte, each said solid-
3 phase assay reagent comprising a binding species that is selective active in a single assay

4 and coupled to one of a plurality of microparticles of magnetically responsive material,
5 the sizes of said microparticles varying in size over a range that is an aggregate of a
6 plurality of subranges, each subrange distinguishable from other subranges of said
7 aggregate by flow cytometry and by the binding species coupled thereto.

1 22. A composition in accordance with claim 21 in which said range is a
2 diameter from about 0.3 micrometers to about 100 micrometers.

1 23. A composition in accordance with claim 21 in which said range is a
2 diameter of from about 0.5 micrometers to about 40 micrometers.

1 24. A composition in accordance with claim 21 in which the standard
2 deviation of the particle diameters of each said subrange is less than one third of the
3 separation of the mean diameters of adjacent subranges.

1 25. A composition in accordance with claim 21 in which said
2 microparticles have a porosity substantially less than macroporous.

1 26. A composition in accordance with claim 21 consisting essentially
2 of from two to 100 binding species, each selectively active in a single assay relative to the
3 remaining binding species.

1 27. A composition in accordance with claim 21 in which said
2 microparticles are comprised of a combination of a polymer and a paramagnetic
3 substance.

1 28. A composition in accordance with claim 27 in which said
2 paramagnetic substance is a metal oxide.

1 29. A composition in accordance with claim 27 in which said polymer
2 is formed from monomers including carboxylate groups to permit covalent bonding of
3 assay binding members at the microparticle surface.

1 30. A method for the analysis of a sample to simultaneously yet
2 individually detect antibodies of different classes that have a single common antigen
3 specificity, said method comprising:

contacting said sample with a first solid phase coated with said antigen to cause antibodies in said sample to bind to said antigen;

washing said first solid phase to isolate said first group from unbound species;

suspending said first solid phase thus washed in a liquid suspending medium and releasing said antibodies from said antigen into said medium to form a supernatant containing unbound multiple-class antibodies specific to said antigen;

isolating said supernatant from said first solid phase and contacting said supernatant with a second solid phase, said second solid phase comprising one or more portions each coated with an immunological binding member with specific binding affinity for a single antibody class, and each portion being capable of differentiation from other such portions; and

individually detecting the occurrence of immunological binding between said antibody classes and said immunological binding members while differentiating between said portions.

1 31. A method in accordance with claim 30 in which said first solid
2 phase is a plurality of microparticles.

1 32. A method in accordance with claim 30 in which said second solid
2 phase is a plurality of microparticles.

1 34. A method in accordance with claim 30 in which said occurrence of
2 immunological binding is detected using labeled class-specific antibodies.

1 35. A method in accordance with claim 30 in which said occurrence of
2 immunological binding is detected using phycoerythrin-labeled class-specific antibodies
3 and individual detection is achieved by fluorescence detection.

1 36. A method in accordance with claim 30 in which said occurrence of
2 immunological binding is detected using labeled analogs of said antigen.

1 37. A method in accordance with claim 30 in which said occurrence of
2 immunological binding is detected using phycoerythrin-labeled analogs of said antigen
3 and individual detection is achieved by fluorescence detection.

1 38. A method in accordance with claim 31 in which said microparticles
2 are of magnetically responsive material, and said washing is facilitated by magnetically
3 separating said first group of microparticles from said sample.

1 39. A method in accordance with claim 31 in which said microparticles
2 are of magnetically responsive material, and both said washing of said first group of
3 microparticles and said isolating of said supernatant from said first solid phase are
4 facilitated by magnetically separating said microparticles from said sample and said
5 supernatant.

1 40. A method in accordance with claim 32 in which said portions are
2 subgroups of microparticles differing from each other by particle size, and differentiation
3 between said subgroups is achieved by flow cytometry.

1 41. A method for the analysis of a sample to simultaneously yet
2 individually detect antibodies of different antigen specificities that are of a single
3 common immunoglobulin class, said method comprising:

4 contacting said sample with a first solid phase coated with class-specific
5 antibody having specific binding affinity for a single selected class of antibodies
6 to cause antibodies in said sample of said single selected class to be immobilized
7 to said first solid phase through immunological binding to said class-specific
8 antibody coated thereon;

9 washing said first solid phase to isolate said first solid phase from unbound
10 species;

11 contacting said first solid phase thus washed with a liquid medium and
12 releasing said antibodies of said single selected class from said first solid phase by
13 dissociating said immunological binding to form a supernatant containing
14 antibodies of a said selected class but of multiple antigen specificities;

15 isolating said supernatant from said first solid phase and contacting said
16 supernatant with a second solid phase, said second solid phase comprising one or

17 more portions each coated with an immunological binding member with specific
18 binding affinity for antibodies of a single antigen specificity that is distinct from
19 antigen specificities of other portions, and each portion being capable of
20 differentiation from other such portions; and

21 individually detecting the occurrence of immunological binding between
22 said antibodies and said immunological binding members while differentiating
23 between said portions.

1 42. A method in accordance with claim 41 in which said first solid
2 phase is a plurality of particles.

1 43. A method in accordance with claim 41 in which said second solid
2 phase is a plurality of particles.

1 44. A method in accordance with claim 41 in which said first and
2 second solid phases are each a plurality of particles.

1 45. A method in accordance with claim 42 in which said microparticles
2 are of magnetically responsive material, and said washing is facilitated by magnetically
3 separating said microparticles from said sample.

1 46. A method in accordance with claim 42 in which said microparticles
2 are of magnetically responsive material, and both said washing of said microparticles and
3 said isolating of said supernatant from said microparticles are facilitated by magnetically
4 separating said first group of microparticles from said sample.

1 47. A method in accordance with claim 43 in which said portions differ
2 from each other by particle size, and differentiation between said portions is achieved by
3 flow cytometry.

1 48. A method in accordance with claim 43 in which said individual
2 detection of the occurrence of immunological binding is achieved by the use of
3 fluorophore-labeled binding members.

1 49. A method in accordance with claim 48 in which said fluorophore-
2 labeled binding members are phycoerythrin-labeled binding members.